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WANTED – Dead or alive: Myotubularins, a large disease-associated protein family

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ABSTRACT

Myotubularins define a large family of proteins conserved through evolution. Several members are mutated in different neuromuscular diseases including centronuclear myopathies and Charcot-Marie-Tooth (CMT) neuropathies, or are linked to a predisposition to obesity and cancer. While some members have phosphatase activity against the 3-phosphate of phosphoinositides, regulating the phosphorylation status of PtdIns3*P* and PtdIns(3,5)P_2 implicated in membrane trafficking and autophagy, and producing PtdIns5*P*, others lack key residues in the catalytic site and are classified as dead-phosphatases. However, these dead phosphatases regulate phosphoinositide-dependent cellular pathways by binding to catalytically active myotubularins. Here we review previous studies on the molecular regulation and physiological roles of myotubularins. We also used the recent myotubularins three-dimensional structures to underline key residues that are mutated in neuromuscular diseases and required for enzymatic activity. In addition, through database mining and analysis, expression profile and specific isoforms of the different myotubularins are described in depth, as well as a revisited protein interaction network. Comparison of the interactome and expression data for each myotubularin highlights specific protein complexes and tissues where myotubularins should have a key regulatory role.

Contents

Abbreviations: MTM, Myotubularins; PPIn, phosphoinositides; PtdIns, phosphatidylinositol; CNM, centronuclear myopathy; CMT, Charcot-Marie-Tooth neuropathy; PH-GRAM, Pleckstrin Homology, Glucosyltransferase, Rab-like GTPase Activator and Myotubularins; FYVE, Fab1-YOTB-Vac1-EEA1; NFL, neurofilament light chain.

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1. Introduction

Myotubularins constitute a large disease-associated family highly conserved through evolution with similarities to phosphatases. In humans there are 14 clear paralogs of myotubularins: the first identified was MTM1 followed by 13 myotubularin-related proteins MTMR1 to MTMR13 (Laporte et al., 1996, 2003; Robinson and Dixon, 2006). Among them, 8 proteins are active phosphatases while 6 are catalytically dead, with a functional cooperation between members of these two classes (Kim et al., 2003; Nandurkar et al., 2003). In addition MTMR14 protein (also named hJUMPY) has been described (Tosch et al., 2006), however phylogenetic studies and protein domain composition suggested it defines a close but distinct protein family, and therefore this protein will not be discussed further in this review. Additional pseudogenes related to myotubularins also exist (Alonso et al., 2004).

Although active myotubularins have been tentatively classified as Protein Tyrosine Phosphatases (PTP) based on the presence of a $C(X)_5R$ motif, they are specific phosphoinositides (PPIn) 3-phosphatases that dephosphorylate the phosphatidylinositol-3-monophosphate (PtdIns3*P*) and PtdIns(3,5)P_2 into PtdIns and PtdIns5*P*, respectively (Blondeau et al., 2000; Taylor et al., 2000; Tronchere et al., 2004; Walker et al., 2001). Conversely, dead myotubularins share a similar organization in domains but lack the phosphatase activity (Cui et al., 1998; Nandurkar et al., 2001). PPIn are lipid second messengers implicated in a wide range of cellular processes from cell growth and survival to cytoskeleton dynamics (Di Paolo and De Camilli, 2006; Staiano et al., 2015). More specifically, PtdIns3*P* and PtdIns(3,5)P_2 regulate membrane trafficking at the endosomal level and autophagy, which are the most studied and characterized functions of myotubularins (Nicot and Laporte, 2008; Robinson and Dixon, 2006). PtdIns5*P* is implicated in several cellular processes including oxidative stress signaling, growth factor signaling and transcriptional regulation (Bulley et al., 2015; Giudici et al., 2016; Gozani et al., 2003; Keune et al., 2013; Ramel et al., 2011).

Myotubularins have been found in almost all eukaryotes from yeast to mammals, with few exceptions, such as *P. falciparum* (Lecompte et al., 2008). Orthologs for the 14 human myotubularins are found in chordates, except in rodents where MTMR8 is absent at least in mice and rats. A co-evolution has been observed between active and dead myotubularins, as well as between active myotubularins and antagonist kinases (Lecompte et al., 2008); for example MTM1 with the class-III PtdIns 3-kinase VPS34 (PIK3C3) and its regulator VPS15 (PIK3R4). Why have so many myotubularins been duplicated and conserved? Indeed, the presence of 14 similar proteins in humans could lead to functional redundancy, however this high evolutionary pressure suggests that each myotubularin has one or several specific function(s). This specificity could be related to tissue expression or splice isoforms, or particular protein-protein interactions. This specific point will be developed in this review.

To date, mutations were found in 3 myotubularin genes in monogenic human diseases. *MTM1* is mutated in X-linked centronuclear myopathy (XLCNM, OMIM: 310400) also called myotubular myopathy, characterized by hypotonia at birth, a very severe and generalized muscle weakness, external ophthalmoplegia and respiratory distress (Jungbluth et al., 2008; Laporte et al., 1996). Two other myotubularins, *MTMR2* (encoding active phosphatase) and *MTMR13/SBF2* (dead phosphatase), are mutated in Charcot-Marie-Tooth neuropathy type 4B1 (4B1, OMIM: 601382) and 4B2 (CMT4B2, OMIM: 604563), respectively (Azzedine et al., 2003; Bolino et al., 2000; Senderek et al., 2003). CMT4B1 and 2 are two distinct but close forms of autosomal recessive demyelinating neuropathy affecting peripheral nerves and leading to pronounced muscular atrophy and weakness of distal limbs. In addition, several myotubularins are linked to multifactorial diseases as colorectal, gastric and lung cancers (MTMR3 and 7) (Hu et al., 2011; Song et al., 2010; Weidner et al., 2016), obesity (MTMR9) (Hotta et al., 2011) and Creutzfeldt–Jakob disease (MTMR7) (Sanchez-Juan et al., 2012). The fact that ubiquitously expressed myotubularins are implicated in different tissue-specific diseases again indicates that the apparent biochemical redundancy is in fact hiding tissue-specific functions.

This review focuses on recent advances concerning 3 main aspects of the myotubularin family: gene expression, protein interactions and protein structure. Through database mining and analysis, the interaction network of myotubularins is revisited and integrated, and their expression profiles and specific isoforms are described.

2. Myotubularins: protein domains and interactions

Myotubularins are multidomain proteins that share the same central core composed of the PH-GRAM (Pleckstrin Homology - Glucosyltransferase, Rab-like GTPase Activator and Myotubularins) domain that could bind to PPIn or proteins and the phosphatase-like domain (Fig. 1A) (Begley et al., 2003; Choudhury et al., 2006; Doerks et al., 2000; Tsujita et al., 2004). In the 8 active myotubularins (MTM1, MTMR1-4 and 6–8), the catalytic domain contains the consensus $C(X)_5R$ signature motif (Alonso et al., 2004; Zhang et al., 1994). In the 6 phosphatase dead myotubularins (MTMR9-12, MTMR5/Sbf1 and MTMR13/Sbf2), the absence of enzymatic activity is due to the substitution of catalytically essential residues such as the cysteine in the consensus motif (Cui et al., 1998; Nandurkar et al., 2003).

Myotubularins can have several other functional domains: the PDZ binding site (MTM1, MTMR1 and 2) mediates protein-protein interactions, the PH (Pleckstrin homology) (MTMR5 and 13) and FYVE (Fab1-YOTB-Vac1-EEA1) (MTMR3 and 4) domains can bind PPIn (Itoh and Takenawa, 2002), and the DENN domain (MTMR5 and 13) is involved in small Rab GTPase regulation (Fabre et al., 2000; Jean et al., 2012; Yoshimura et al., 2010). By combining domain organization and phylogenetics, 6 different subgroups are highlighted, each containing exclusively active or dead members (Fig. 1B). In addition, all myotubularins except MTMR10 contain a coiled-coil (CC) domain that is critical for their homodimerization and/or heterodimerization (Berger et al., 2006; Lorenzo et al., 2006). Dimerization also appears to depend on the PH-GRAM domain (Berger et al., 2006).

Indeed, all myotubularins except MTMR11 have been shown to interact either with themselves or with other myotubularins (Fig. 1C) (Berger et al., 2006; Gupta et al., 2013; Kim et al., 2003; Lorenzo et al., 2006; Mochizuki and Majerus, 2003; Nandurkar et al., 2003; Schaletzky et al., 2003; Zou et al., 2009). Within the 14 members, 9 have been reported to form homodimers; this could enhance the membrane targeting by coupling two PH-GRAM domains (Berger et al., 2003). At least for MTM1, homo-oligomerization controls its allosteric activity, and *in vitro* MTM1 incubated with its substrate PtdIns3*P* forms a heptamer in the presence of PtdIns5*P* (Schaletzky et al., 2003). One of the most notable characteristics of this family is that most heterodimers are formed by a coupling between active and dead phosphatases. For example, MTMR2 forms heterodimers with its dead homologs MTMR5/Sbf1, MTMR10, MTMR12 and MTMR13/Sbf2. The fact that mutations in MTMR13 lead to a similar neuropathy (CMT4B) as defects in MTMR2 confirms the physiological importance of dead phosphatases and heterodimers. MTMR9 interacts with 3 different active myotubularins of the same phylogenic subgroup (MTMR6, 7 and 8)

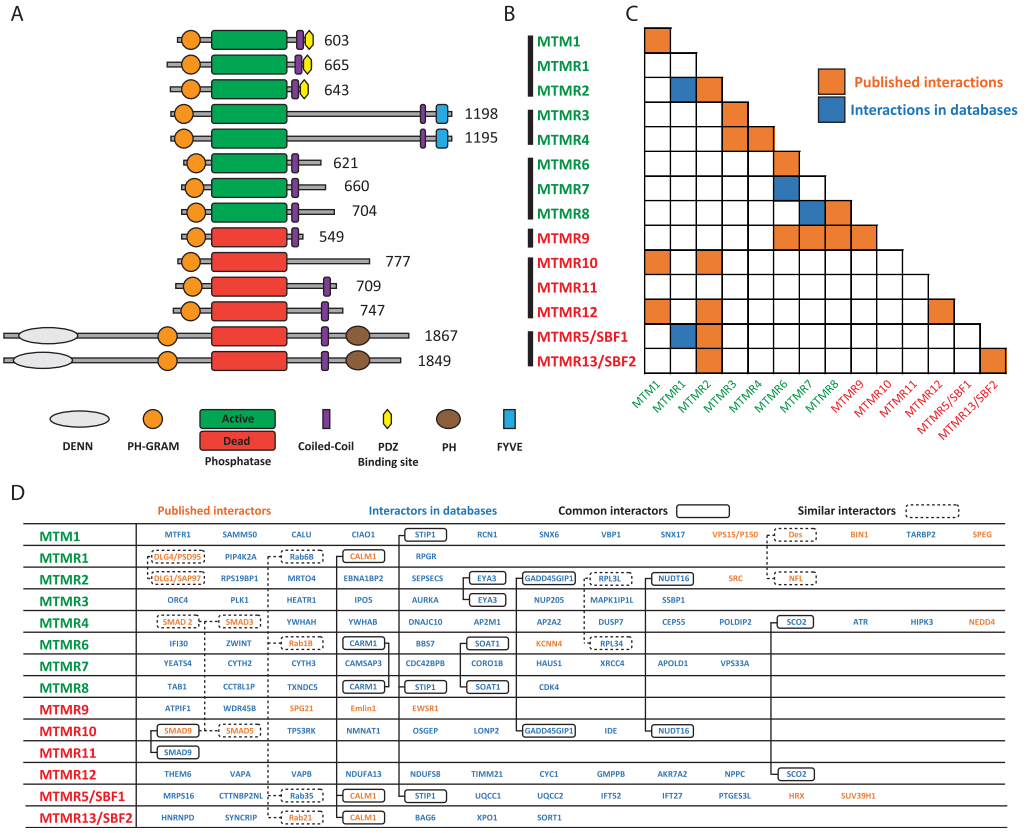

Fig. 1. Human myotubularins: domain organization and interactome. A. Scaled representation of the protein domains of human myotubularins. All myotubularins share the PH-GRAM and phosphatase (active or dead) domains. For each myotubularin, amino acid length of the most described protein isoform is indicated. **B**. Classification of myotubularins into 6 subgroups based on protein organization and phylogenetics (indicated by the vertical bars on the left). Active myotubularins are represented in green and dead myotubularins in red. **C**. Known protein interactions within the myotubularin family. Published interactions are in orange while interactions found in databases (Biogrid - the biogrid.org) are in blue. **D**. List of known interactors for each myotubularin. Published interactors are represented in orange while interactors found in databases with a minimum MUSEscore of 0,95 (Biogrid and Li et al., 2016) are in blue. Common interactors and interactors of the same protein family are surrounded and related together by a continuous and stippled line, respectively. Similar interactors found for a specific myotubularin are not surrounded.

and increases their enzymatic activity (Mochizuki and Majerus, 2003). In the same way, MTMR5 increases the enzymatic activity of MTMR2 and both are related to male infertility due to impaired spermatogenesis (Bolino et al., 2004; Firestein et al., 2002; Kim et al., 2003). In some heterodimers, such as MTM1-MTMR12, MTMR2-MTMR5 and MTMR2-MTMR13, the dead myotubularin may regulate the cellular localization of the active member (Gupta et al., 2013; Kim et al., 2003; Nandurkar et al., 2003). Dead myotubularins appear early in evolution and are conserved in many species (Laporte et al., 2003; Lecompte et al., 2008). Altogether, this suggests (1) a co-evolution between active and dead myotubularins and (2) that myotubularins rely on oligomerization for their function since very early in the evolution. Heterodimers can also be formed between two active members of the same phylogenic subgroup, as for MTMR1-MTMR2, MTMR3-MTMR4, MTMR6-MTMR7 and MTMR7-MTMR8 (Fig. 1C).

Numerous interactors have been identified for each myotubularin (Fig. 1D) (Biogrid - thebiogrid.org and Intact - http://www.ebi.ac.uk/intact/) (Agrawal et al., 2014; Berggard et al., 2006; Cao et al., 2007; Cui et al., 1998; Fabre et al., 2000; Firestein et al., 2000; Jean et al., 2012; Li et al., 2016; Plant et al., 2009; Royer et al., 2013; Rual et al., 2005; Srivastava et al., 2005; Yu et al., 2013; Zhang et al., 2005). Some myotubularins share common interactors or interactors from the same protein family. For example, MTMR6 and MTMR8 both interact with SOAT1, a protein localized in the endoplasmic reticulum, which is also the presumed localization of these myotubularins. MTM1 interacts with desmin and MTMR2 with neurofilament light chain (NFL), that are two intermediate filament proteins specifically found in muscles and neurons, respectively (Hnia et al., 2011; Previtali et al., 2003). This is consistent with mutations in MTM1 and desmin leading to myopathies and mutations in MTMR2 and NFL leading to CMT neuropathies (Goldfarb et al., 1998; Mersiyanova et al., 2000). Another well-represented group of interactors is the Rab family: MTMR1-RAB6B, MTMR6-RAB1B, MTMR5-RAB35 and MTMR13-RAB21 (Jean et al., 2012; Mochizuki et al., 2013). Rabs constitute a very large GTPase family regulating many steps of membrane trafficking, one of the main cellular functions in which myotubularins are implicated (Barr, 2013). Of note, myotubularins implicated in 3 heterodimers share common or similar interactors: MTMR1-MTMR2, MTMR1-MTMR5 and MTMR2-MTMR10. For example, MTMR1 and MTMR5 heterodimerize and interact with different Rab GTPases (Fig. 1D).

3. Myotubularins: tissue expression

To investigate how myotubularin genes are expressed in human tissues, we mined the Genotype-Tissue Expression (GTEx) database, which has been built by systematic RNA-sequencing using samples of 51 different tissues from hundreds of donors and 2 transformed cell types in culture. Fig. 2 shows for each gene the relative expression in all tested tissues, and highlights in which tissue a specific myotubularin is the most/less expressed. This is not absolute expression, therefore a gene cannot be directly compared to another for the same tissue. A dendrogram was generated using the Pearson correlation coefficient to highlight hierarchical clustering of myotubularins sharing similar profiles of expression. Whilst this is one of many possible dendrograms and thus it has to be interpreted cautiously, two main groups of myotubularins can be distinguished based on expression profiles: MTMR1-3-8-11-12-13 (upper branch, Fig. 2), and the others (lower branch). Discriminant tissues between the two groups are brain (almost all regions), skin, vagina and prostate. This does not seem to be directly related to phylogenetic classification, to active/dead and active/active heterodimers or to myotubularins sharing common interactors. However, some links can be made. For example, MTMR7 and MTMR9 that form an active/dead heterodimer have the closest expression profiles and are both strongly expressed in brain tissues. A similar link applies to MTM1, MTMR2 and MTMR10, which have correlated expression patterns: they form two active/dead heterodimers MTM1/MTMR10 and MTMR2/MTMR10, and MTMR2 and MTMR10 have common interactors (Fig. 1C and D) (Lorenzo et al., 2006).

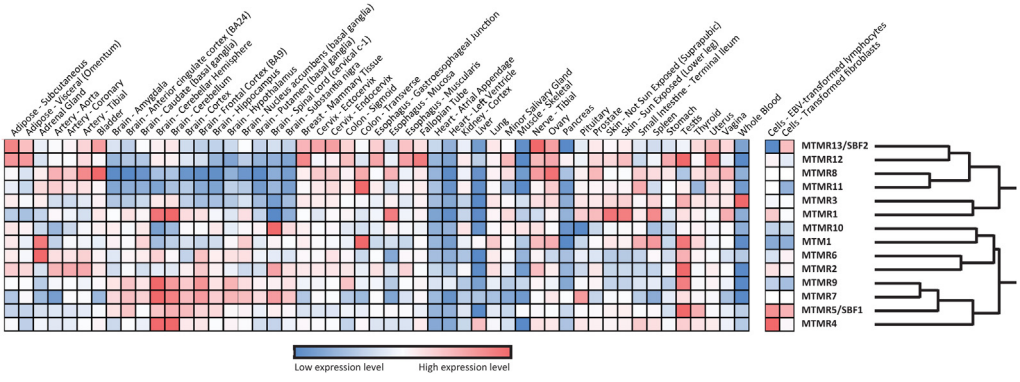

Fig. 2. Myotubularins tissue expression. Heatmap of myotubularin genes relative expression within 51 different tissues underlining in which tissue a specific myotubularin is the most/least expressed (left panel), and absolute expression in 2 cell types. Expression levels have been obtained by mining the GTEx database (www.gtexportal.org/home/). Dendrogram highlighting the hierachical clustering of myotubularins (right panel), using the Pearson correlation coefficient and average linkage.

Concerning myotubularins related to monogenic diseases, while MTM1 has a low expression level in striated muscles compared to other tissues such as nerves, colon or testis, mutations in the *MTM1* gene lead to a severe myopathy. Thus, the MTM1 tissue-specific function could be explained by interactions with partners that are only expressed in muscle, such as desmin (Hnia et al., 2011). On the contrary, MTMR2 and MTMR13 are highly expressed in nerves, which is consistent with the neuropathies observed due to mutations in these genes. In addition, MTMR2 binds the neuronal intermediate filament NFL (Previtali et al., 2003), highlighting a potential molecular basis common to different CMT neuropathy forms. A link can be observed between MTMR2 and MTMR5, known to form heterodimers; they both have a high relative expression level in testis, and defects of these genes lead to male infertility by impaired spermatogenesis (Bolino et al., 2004; Firestein et al., 2002), therefore adding weight to the physiological significance of this data.

Myotubularins expression levels have also been measured in two cell types, lymphocytes and fibroblasts, that are easily derived from human cells. These cells could allow diagnosis at the protein level or be dedifferentiated into induced pluripotent stem (IPS) cells that could be reprogrammed into specific cell types, allowing study of the pathocellular mechanisms. This time, absolute expression levels of all myotubularins are compared (Fig. 2). Some myotubularins, such as MTMR5 or MTMR2, are well expressed in both lymphocytes and fibroblasts, whereas for other myotubularins fibroblasts show a higher expression level, as for MTMR13 for which study in these cell types might be more adapted. Therefore, interpreting this data can be useful in deciding which cell lines should be used for research and diagnostic purposes.

4. Myotubularin: mRNA isoforms

The study of gene expression does not take splicing events into account. Indeed, a specific gene is often spliced into several mRNA isoforms that could be translated into different protein isoforms. In this review, we use the term “isoform” to define a variant of the same protein or mRNA, and “homologs” for different genes. Fig. 3 summarizes the myotubularin mRNA isoforms expression within all tissues present in the GTEx database. Only significantly expressed mRNA isoforms have been represented, and color-coded based on their predicted protein product: the main protein isoform from the literature, longer/shorter protein isoforms, or non-coding mRNA isoforms. For each mRNA isoform, the expression level is indicated as a percentage of total gene expression.

Interestingly, the main mRNA isoform studied in the literature is not always the most expressed (MTM1, MTMR3, MTMR10, MTMR11 and MTMR12). For MTMR10, the most expressed isoform encodes only the PH-GRAM; it raises the possibility that this protein isoform exerts a dominant negative effect on oligomerization of myotubularins or on cellular functions. MTMR2 has 4 well expressed mRNA isoforms: one translated into the main 643 amino acids (aa) protein isoform and the three others translated into a 571 aa protein isoform lacking the N-terminal extremity before the PH-GRAM (Bolino et al., 2001). The latter is present in all tissues except brain, and may have a specific function. In addition, some isoforms are tissue-specific, as for MTM1 with 2 isoforms only expressed in skeletal muscle. Corresponding peptides lack a part of the PH-GRAM domain and could support a muscle-specific function altered in the MTM1-related myopathy. In total, 10 myotubularins express mRNA isoforms leading to shorter proteins and MTMR1 displays an isoform predicted to encode a longer protein. These differences can affect various protein domains as the FYVE domain for MTMR3 and MTMR4 or the DENN domain for MTMR5, and thus could highly impact on protein conformation or protein-protein/protein-lipid interactions.

For MTM1, MTMR11 and MTMR13, the prevailing mRNA isoforms are non-coding, or the corresponding peptides have not been identified yet, questioning the function of such isoforms. Finally, some isoforms described in the literature are not represented here because they were absent in the GTEx database. This is the case for various MTMR1 mRNA isoforms that are known to be well expressed in some tissues (Buj-Bello et al., 2002).

In the future, it would be important to characterize the cellular activity of these tissue-specific isoforms, in order to get insight into their physiological relevance.

5. Myotubularins: protein structure

Between 2003 and 2016, the crystal structures of 4 active myotubularins have been determined: MTMR1 (PDB: 5C16), MTMR2 (1LW3, 1ZVR, 1M7R and 1ZSQ), MTMR6 (2YFO) and MTMR8 (4YZI) (Begley et al., 2003, 2006; Bong et al., 2016). A crystal structure of mouse MTMR5 has also be resolved, but only contains the C-terminal PH domain (1V5U). No major differences have been described between the 4 structures, except for the orientation of the MTMR6 PH-GRAM domain; this could impact MTMR6 oligomerization properties (Bong et al., 2016). From a 3D point of view, myotubularins are globular proteins with two main parts: the PH-GRAM domain and the catalytic domain, connected by a linker (Fig. 4A) (Begley et al., 2003). N-terminal extremities, coiled-coil domains and PDZ binding sites are absent of these structures, presumably because they are too flexible or cleaved by proteolysis. In addition, the cysteine residue of the catalytic $C(X)_5R$ motif has been mutated into a serine for crystallization, except for MTMR6.

The PH-GRAM domain consists in 7 β-strands and 1 α-helix. The main characteristic of the catalytic domain is the substrate binding pocket that is significantly deeper and wider than that of classical tyrosine phosphatases, explaining the unique specificity of myotubularins for membrane-embedded PPIn substrates (Fig. 4A) (Begley et al., 2003). Indeed, active myotubularins specifically hydrolyze the D-3 position of PtdIns3*P* and PtdIns(3,5)P_2, two PPIn with large phosphorylated inositol headgroups that perfectly fit in the catalytic pocket. The D-3 phosphate is then trapped by its interaction with the main chain of 6 residues from the $C(X)_5R$ motif loop (on MTMR2 sequence: Cys417, Ser418, Gly420, Trp421, Asp422 and Arg423) (Fig. 4B).

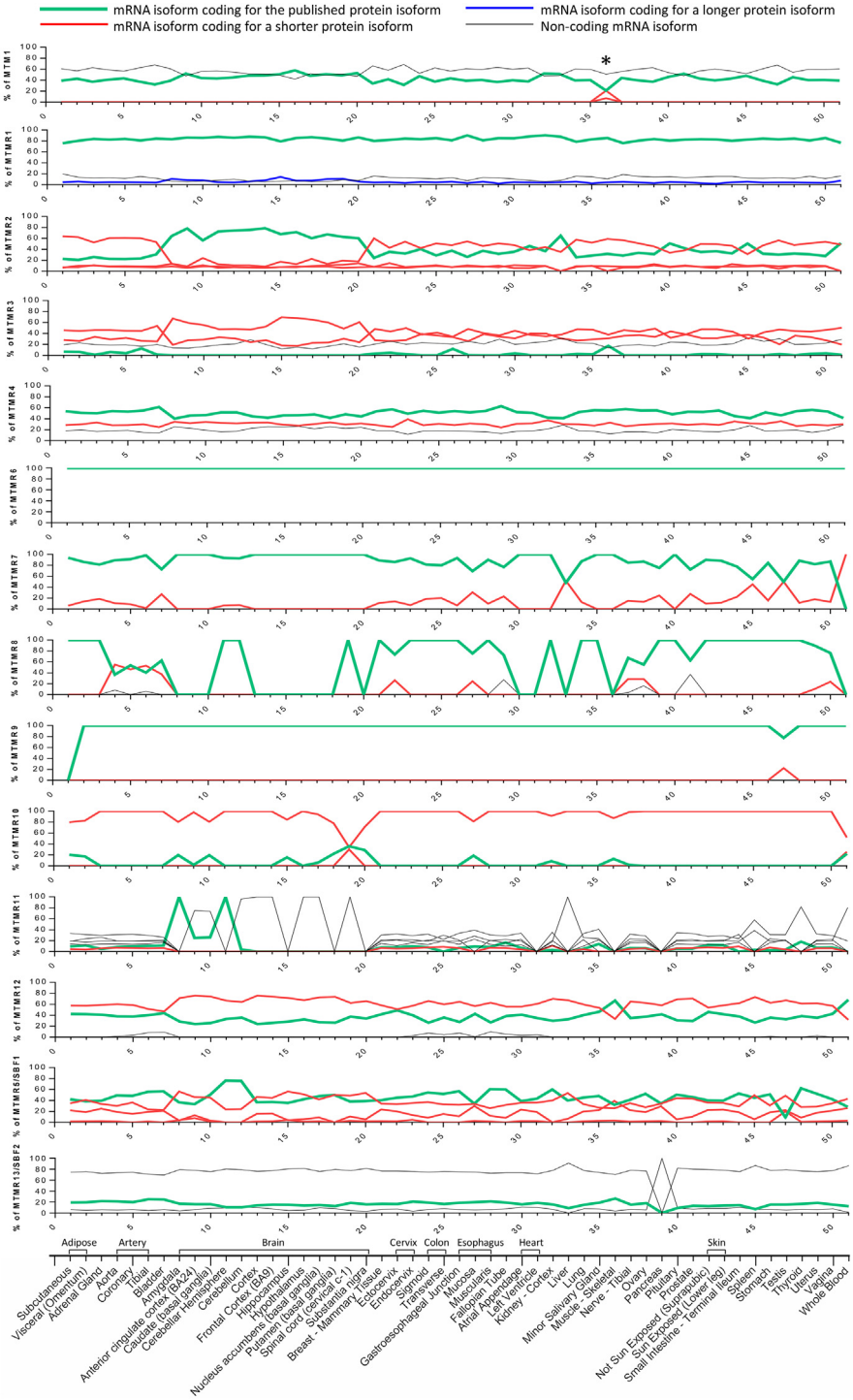

Fig. 3. Myotubularin mRNA isoforms. For each myotubularin indicated on the left, the most expressed isoforms present in the GTEx database are represented as a percentage of total gene expression. Only the most expressed isoforms are shown. The mRNA isoforms coding for the main published protein isoforms are indicated in green, shorter isoforms in red and longer isoforms in blue. Several non-coding isoforms indicated in black have been found well expressed, for which no corresponding peptides have been described yet. The star indicates specific MTM1 mRNA isoforms in skeletal muscle, the tissue affected in MTM1-related myopathy. For several myotubularins as MTMR3, MTMR10 and MTMR12, and to a less extend MTMR2, the main expressed isoforms are different than the published isoforms used for functional characterization of the related proteins; for MTMR11 and MTMR13 the main expressed isoforms are predicted non-coding.

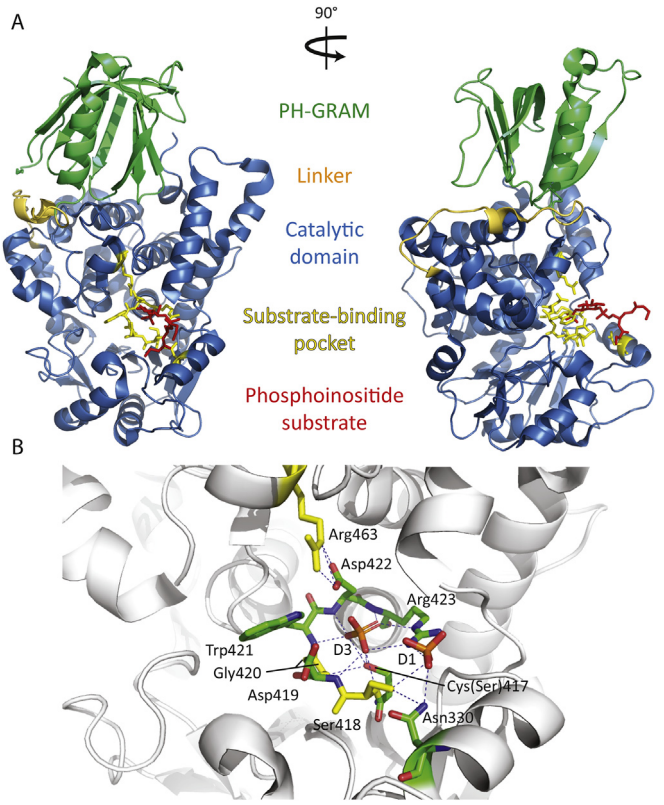

Fig. 4. The myotubularins protein structure. A. Overall view of the myotubularin structure. The crystal structure of MTMR2 (PDB:1ZSQ) is used as a model, with a front view and a view rotated at 90°. Domain names and the phosphoinositide substrate (here PtdIns3*P*) are indicated on the two representations. **B**. Zoom on the substrate-binding pocket. Residues forming the $C(X)_5R$ loop and other important residues are represented using stick models. Residues affected by missense mutations in MTM1-linked centronuclear myopathy are colored in yellow. No missense mutations have been found in *MTMR2* or *MTMR13* genes in the catalytic pocket. The cysteine residue of the $C(X)_5R$ motif is mutated to serine in the structure for crystallization purposes. Hydrogen bonds between the two phosphate groups in position D1 and D3 of PtdIns3*P*/PtdIns(3,5)P_2 and surrounding residues of the active site are represented by stippled lines.

Concerning the catalytic activity, the nucleophile Cys417 residue attacks the phosphorous atom in position D-3 of the PPIn substrate, forming a phosphoenzyme intermediate, then the aspartic acid (Asp422 in MTMR2) donates a proton to the released dephosphorylated substrate, before hydrolysis yielding free enzyme and inorganic phosphate (Begley and Dixon, 2005; Begley et al., 2006; Nandurkar and Huysmans, 2002). Myotubularins are different from classical PTPs because the catalytic aspartate residue lies directly in the catalytic $C(X)_5R$ loop and not in a WPD-loop. The D-1 phosphate of the PPIn interacts with the side chain of two residues from the $C(X)_5R$ motif (on MTMR2 sequence: Ser418 and Arg423), but also with Asn330, which is conserved in all active myotubularins suggesting an important role in PPIn substrate binding. Some other residues help to maintain the three-dimensional structure of this catalytic pocket, like Arg463 (on MTMR2) that is also conserved in all myotubularins. A phosphate in position D-4 would generate a steric clash with several residues of the catalytic pocket, excluding PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 from potential substrates.

Another consideration for myotubularin substrate specificity is that active myotubularins are electrostatically polarized proteins, with one strongly electropositive side containing the catalytic site (Begley et al., 2006). This would create electrostatic interactions between the positively charged face of myotubularins and the negatively charged membranes containing PPIn, contributing to substrate-binding affinity. This electropositive patch around the catalytic pocket seems to be specific for active myotubularins, while several dead myotubularins have an electronegative surface, suggesting a poor affinity toward lipid membranes. Furthermore, active myotubularins could bind either membranes or dead-phosphatase homologs through the same interface.

The three-dimensional structure can also be very useful to understand the effect of disease-associated mutations and thereby to evaluate the importance of mutated residues for the function or the stability of the protein. The majority of MTM1, MTMR2 and MTMR13 missense mutations affect residues in the hydrophobic core structure of the PH-GRAM and catalytic domains, and replace native amino acids by bulkier residues, or decrease van der Waals contacts or alter internal hydrogen bonds, consequently disrupting the protein core structure. In addition, two clusters of solvent-accessible

missense mutations at the surface of the MTM1 protein can be observed: the Pro179-Asn180-Arg184 cluster and the Asp431-Asp433 cluster (numbered in MTM1 sequence) that could be potential binding sites for interactors (Begley et al., 2003). In the active site, the Ser376, Gly378 and Arg421 (numbered Ser418, Gly420 and Arg463 in MTMR2 structure in Fig. 4B) are frequent sites of mutations found in MTM1: the Ser376 and Gly378 directly bind the D-3 phosphate of the PPIn and the Arg421 is a key factor to maintain the position of the catalytic loop. Thus, mutations of these residues would directly prevent any catalytic activity.

6. Conclusion

Myotubularins define a large and highly conserved family of proteins with some noteworthy characteristics. They are classified in the Protein Tyrosine Phosphatases (PTP) family but have a specific phosphatase activity against phosphoinositides. One other feature is the presence of catalytically active and dead phosphatases, where dead myotubularins regulate their active homologs. Although they are ubiquitously expressed, three myotubularin genes – *MTM1*, *MTMR2* and *MTMR13* – are mutated in tissue-specific neuromuscular diseases, suggesting tissue-specific splice isoforms or specific protein-protein or protein-lipid interactions.

Future experiments will be needed to address this tissue specificity. While the function of myotubularins and PPIn substrates and products was well studied in cell systems, their physiological role *in vivo* is still barely understood. Another key issue is the pathological mechanism(s) associated to myotubularin-related diseases. Data showed that MTM1-related myopathy or phenotypes can be rescued in mice and drosophilia by inhibition or muscle-specific ablation of the class II PtdIns 3-kinase, pointing to the importance of PPIn regulation by myotubularin (Sabha et al., 2016; Velichkova et al., 2010). However, the same mouse disease model can also be rescued by expressing a phosphatase inactive MTM1 protein, supporting that PPIn-unrelated functions of myotubularin are implicated in this pathology (Amoasii et al., 2012). Due to the importance of myotubularins and PPIn pathways in metabolism and cellular integrity, it is expected that their dysregulation in more common diseases will be highlighted in the future.

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